<u>In the specification</u>:

At page 1, after the title, please enter the following:

-- <u>RELATED APPLICATION</u>

The present application is a divisional application of U.S. application Ser. No. 10/223,978, filed August 20, 2002, which claims priority to Korean Application 10-2001-0072486, filed November 20, 2001. --

Please substitute the following paragraph at page 45, line 14 – page 46, line 8:

-- **Example 2:Bacterial expression and purification of GST-synuclein fusion proteins**

The expression vectors constructed in Example 1 for expression of GST-synuclein fusion proteins were transformed into the *E. coli* strain, BL21 (DE3) plysS (Invitrogen). The transformed bacteria were grown in a LB medium containing 0.1 mg/ml ampicillin at 37℃ to an A_{600} of 0.8, induced with 0.5 mM IPTG and then, cultured for a further 4 hours. The culture was then centrifuged at 10,000 rpm for 10 minutes to harvest cells. The cells were resuspended in phosphate-buffered saline (PBS, pH 7.4) and disrupted by ultrasonication. After removing the cell debris, the supernatants were purified by affinity chromatography. That is, the supernatants were passed through a glutathione-Sepharose 4B column (Peptron, Taejeon, Korea) equilibrated with PBS. After washing with PBS, the fusion proteins were eluted with 10 mM GSH (Sigma, St. Louis, MO). The eluted GST-synuclein fusion proteins were further purified on an FPLC gel-filtration column and concentrated by using ~~the Centricon condencer~~ <u>CENTRICON filter</u> (Amicon, Beverly, MA). --

Please substitute the following paragraph at page 55, line 1-23:

-- **Example 8:GST activity of GST-synuclein fusion proteins after heat treatment**

Unlike the wild type GST protein described in the foregoing Examples, GST-synuclein fusion proteins containing the ATS α were found to be heat resistant. This suggests that the heat-labile protein could be transformed into a heat-resistant protein simply by introducing the ATS α . Subsequently, whether or not the heat-resistant GST-fusion proteins could keep the enzymatic activity after heat treatment was investigated. The GST and GST-synuclein fusion proteins were boiled in a water bath for 10 minutes and cooled in the air at room temperature. The enzymatic activities of these heat-treated proteins were then compared. The enzymatic activity was assayed using a chromogenic substrate, 1-chloro-2,4-dinitro benzen (DTNB) (Habig W. H. et al., J. Biol. Chem., 249, 7130-7139 (1974)). The purified GST and GST-synuclein

fusion proteins were diluted into the substrate solution (1 mM GSH and 2 mM DTNB dissolbed in 0.1 M phosphate buffer, pH 7.4) to a final concentration of 20 μg/ml and incubated at 37℃ for 10 minutes. Upon completion of incubation, the enzymatic activity was assayed by measuring absorbance at 350 nm. The absorbance was measured on a ~~Spectramax~~ SPECTRAMAX 250 microplate reader (Molecular Devices, CA, USA). --

Please substitute the following paragraph at page 57, line 3-17:

-- The present inventors analyzed the secondary structural changes of GST due to thermal denaturation by measuring CD spectra of GST and the GST-Synclein fusion protein. The CD spectra were recorded on a ~~Jasco~~JASCO-J715 spectropolarimeter (Jasco International Co. Ltd., Tokyo, Japan) equipped with a temperature control system in a continuous mode. The far-UV CD measurements were carried out over the wavelength range of 190 to 250 nm with 0.5 nm bandwidth, a one second response time and a 10 nm/minute scan speed at 25℃ and 100℃. The spectra shown are an average of five scans that were corrected by subtraction of the buffer signal. The CD data were expressed in terms of the mean residue ellipticity, [θ] ($deg.cm^2.dmol^{-1}$). The protein samples for CD measurements were prepared in 10 mM sodium phosphate buffer, unless otherwise specified, and all spectra were measured in a cuvette with a path length of 0.1 cm. --

Please substitute the following paragraph at page 60, line 10 – page 61, line 3:

-- **Example 10:pH- and metal-induced protein aggregation**

The pH-induced aggregation of GST and GST-Syn96-140 was investigated by measuring the turbidity at 65℃ according to time. The measurement of the turbidity was carried out by monitoring the apparent absorbance at 360 nm according to time. Each protein was diluted to a final concentration 0.2 mg/ml in buffers with different pH values. The buffers used were 0.1 M acetate (pH 4.0 and 5.0), 0.1 M citrate (pH 6.0), and 0.1 M Tris-HCl (pH 7.4). The protein solutions diluted in buffers were incubated for 1 hour at room temperature and their apparent absorbance were measured in a ~~Beckman~~ BECKMAN DU650 spectrophotometer (~~DU650,~~ Beckman Coulter, Fullerton, CA). The metal-induced aggregation of GST and GST-Syn96-140 was similarly assessed. Each protein was diluted to a final concentration of 0.2 mg/ml in 20 mM Tris-HCl buffers containing 0 to 1.0 mM of Zn^{2+}, or Cu^{2+}. The protein solutions were incubated for 30 minutes at room temperature and their apparent absorbances at 360 nm were measured. --

Please substitute the following paragraph at page 53, line 2 – 5:

-- [a]pI value was calculated by using ProtParam program ~~(www.expasy.ch)~~.
[b]Hydropathy value was calculated by using ProtParam program ~~(www.expasy.ch)~~. --

Please substitute the following paragraph at page 62, line 21 – page 63, line 13:

-- The expression vector (pDHFR-AST α) was transformed into the *E. coli* strain, BL21 (DE3), for protein expression. The transformed bacteria were grown in a LB medium containing 0.1 mg/ml ampicillin at 37℃ to an A_{600} of 0.8. 0.5 mM IPTG was added to the medium, which was cultured for a further 4 hours. The culture was centrifuged at 10,000 rpm for 10 minutes to harvest cells. The cells were resuspended in phosphate-buffered saline (PBS, pH 7.4), and disrupted by ultrasonication. After removing the lysed strains, the supernatants were loaded onto a Ni-NTA column equilibrated with a loading buffer (50 mM phosphate buffer (pH 8.0) containing 0.3M NaCl and 10 mM imidazole). After washing with the loading buffer, the protein was eluted with 250 mM imidazole in the same buffer. the DHFR-ATS α was further purified on an FPLC gel-filtration column. The purified protein was concentrated and buffer-changed by ~~Centricon~~ <u>CENTRICON filter</u> (Amicon, Beverly, MA). --

Please substitute the following paragraph at page 67, line 8 – page 68, line 11:

-- Also, the thermal behaviors of GST-ATS α deletion mutants were quantitatively analyzed by monitoring absorbance at 360 nm according to time while setting the concentration of each protein at 0.2 mg/ml at 65℃ (Lee G.J. and Vierling E., Method Enzymol., 290, 360-65 (1998); and Horwitz J. Proc. Natl. Acad. Sci. USA 89, 10449-53 (1992)). In the experiment, as shown in Fig. 10C, the OD_{360} of the GST protein drastically increased 2 minutes after heat treatment, and most of the protein had aggregated by 3 minutes. In contrast, the GST-ATS α deletion mutants did not aggregate at all even 10 minutes after heat treatment. Next, the GST-ATS α deletion mutants were qualitatively assayed by monitoring the absorbance at 360 nm while varying the concentration from 0.2 mg/ml to 1.0 mg/ml after heat treatment at 80°C for 10 minutes. As shown in Fig. ~~12D~~ <u>10D</u>, GST-Syn96-140 containing the entire region of ATS α and GST-Syn119-140 containing 22 amino acids of ATS α did not precipitate at all after heat treatment regardless of the concentration, while GST-Syn103-115, GST-Syn114-126 and GST-Syn130-140 containing 11-13 amino acids did not precipitate at all at a low concentration but increasingly aggregated as the concentration was raised. It is noted that the aggregation of protein is proportional to the concentration. Thus, it is demonstrated that the deletion mutants of

the GST-ATS α fusion protein have heat resistance superior to that of wild type GST and the heat resistance interestingly varies according to the length of ATS α . Therefore, optimum effects can be achieved by suitably selecting the length of ATS α according to the size and property of a target protein. --